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Efficacy and Deposit Assessment of Tebufenozide Against Gypsy Moth (Lepidoptera:Lymantriidae)

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ABSTRACT

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In 1994, 1995, and 1996, field trials were conducted to determine the efficacy of tebufenozide (Mimic®, RH- 5992) against gypsy moth, *Lymantria dispar* (L.) and droplet deposition on broadleaved foliage. An aqueous formulation (Mimic 2F) and a commercial aqueous suspension formulation with a vegetable oil added to serve as an antievaporant (Mimic 2LV) were aerially applied with fixed-wing aircraft equipped with flat fan nozzles. A single application at 0.06 lb AI/acre of either formulation was the most effective treatment in suppressing gypsy moth populations and protecting foliage.

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Cover Photo: *Cessna 188B (Ag Truck) applying tebufenozide to control gypsy moth, Lymantria dispar (L.)*

For additional copies of this publication or information concerning Tebufenozide, contact either Richard Reardon in Morgantown, WV at (304)285-1566, e-mail rreardon@fs.fed.us, Winfred McLane in Otis ANG Base, MA at 508-563-9303, or the local Cooperative Extension Service office.

Efficacy and Deposit Assessment of Tebufenozide Against Gypsy Moth (Lepidoptera:Lymantriidae)

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INTRODUCTION

The gypsy moth, *Lymantria dispar* (L.), is the most serious insect pest of eastern broadleaved forests. Over the past 20 years, the Federal and State Cooperative Gypsy Moth Suppression Program has provided funding to control potentially defoliating gypsy moth populations on over 5.0 million acres of eastern forests. During this program, there has been a transition from the more traditional chemical insecticides (e.g., acephate, carbaryl) to the microbial insecticide *Bacillus thuringiensis* var. *kurstaki* (*Btk*), insect growth regulator diflubenzuron (Dimilin®) and the gypsy moth nucleopolyhedrosis virus product Gypchek. All of these insecticides are stomach poisons; however, diflubenzuron may also act as a larvicide or ovicide through contact activity. The most effective treatment in terms of consistent population reduction (greater than 80 percent) and foliage protection (less than 40 percent defoliation) is diflubenzuron applied at 0.03 to 0.06 lb AI/acre for one application. The next effective treatments are *Btk* applied at 24 to 36 BIU/acre for 1 or 2 applications and the gypsy moth nucleopolyhedrosis virus (NPV) at 2×10^{11} OB/acre/appl for 2 applications.

In the early 1990's, there was renewed interest to register an additional entomopathogen or insect growth regulator for use against forest defoliators. Preliminary testing by Rohm and Haas Company (1989) found the insect growth regulator tebufenozide (Mimic®, RH-5992) to be highly effective against forest pests: e.g., hemlock looper, *Lambdina fiscellaria fiscellaria* (Guenee); jack pine budworm, *Choristoneura pinus* Freeman; forest tent caterpillar, *Malacosoma disstria* Hubner; and fall cankerworm, *Alsophila pometaria* (Harris). Efficacy has also been documented for the spruce budworm, *C. fumiferana* (Clemens) (Palli et al. 1995, Cadogan et al. 1997).

Tebufenozide is a nonsteroidal ecdysone agonist that mimics the action of the molting hormone 20-hydroxyecdysone that is found in crustaceans and insects (Rohm and Haas Co., Spring House, PA). Tebufenozide belongs to a group of compounds that promotes a lethal premature molt by direct stimulation of the ecdysteroid receptors (whereas diflubenzuron affects chitin synthesis at the regularly scheduled molt) (Wing et al. 1988). Laboratory evaluation of tebufenozide indicates that for insects, larvae of Lepidoptera are sensitive but Coleoptera, Heteroptera or Orthoptera are much less sensitive (Smagghe and Degheele 1994).

There is minimal data concerning the persistence of tebufenozide in coniferous forests (Sundaram 1994; Sundaram et al. 1996a,b; Sundaram et al. 1997a,b) and even less data in broadleaved forests. Nevertheless, it is assumed that the persistence of tebufenozide is similiar to diflubenzuron. In brief, the persistence of the active ingredient diflubenzuron on leaves follows a rapid decline within the first two weeks, after which the remaining diflubenzuron is generally stable on the leaf surface until leaf fall over 4 months after spray (Wimmer et al. 1993). The initial major loss of diflubenzuron from the leaves is due to rain and wind, with subsequent degradation dependent on sunlight, temperature, pH, and microorganism activity. Harrahy et al. (1993) documented the fate of diflubenzuron on leaves when these leaves fall into a stream environment. The persistence of the active ingredients of *Btk* and Gypchek on leaves is less than 14-days, with washoff and degradation by UV wavelengths most important (USDA 1995).

Both Btk and diflubenzuron have documented impacts on selected non-target lepidopteran larvae (Butler and Kondo 1993; Reardon and Wagner 1995; Sample et al. 1993, 1996; Butler et al. 1997a). In addition, diflubenzuron impacts selected aquatic arthropods (Hansen and Garton 1982, Eisler 1992, Fisher and Hall 1992, USDA 1995). Gypchek is specific to gypsy moth (Reardon et al. 1996). Initial reports indicate that tebufenozide applied at field doses had minimal impact on lepidopteran larvae and no significant effect on drift or survival of evaluated amphipods and aquatic insects (Kreutzweiser et al. 1994).

In laboratory studies conducted in 1988, tebufenozide at doses from 0.007 to 0.125 lb AI/acre provided greater than 90 percent mortality of second instar gypsy moth larvae seven days after treatment as well as demonstrated excellent rainfastness on potted red oak, *Quercus rubra*, seedling foliage (McLane and Finney 1991). Efficacy for tebufenozide at 0.03 lb AI/acre was comparable to results obtained for potted seedlings simultaneously treated at the same dose with diflubenzuron. Following ingestion of tebufenozide, a gypsy moth larva stops feeding within 24 hours and produces a new but malformed cuticle beneath the old cuticle.

The larva, unable to shed its old cuticle, dies of dehydration and starvation 2 to 5 days later. Tebufenozide was registered for general forestry use in Canada in 1996 and recently (1999) in the United States.

In the early 1990's, there were several unreplicated field evaluations of tebufenozide against the gypsy moth, although results were inconsistent. This series of studies was conducted to determine the field efficacy and deposit efficiency of two formulations of tebufenozide applied at several doses against gypsy moth in broadleaved forests.

METHODS AND MATERIALS

Field efficacy and deposit analysis studies were conducted over three years (1994, 1995, 1996) in three geographical areas (States of Ohio, Virginia, West Virginia). The efficacy evaluations included: in 1994, a single application of two doses of the Mimic 2F formulation (aqueous flowable); in 1995, a single application of two doses of the Mimic 2F formulation and one dose of the Mimic 2LV formulation (aqueous suspension with a vegetable oil); and in 1996, a single application of one dose of the Mimic 2F formulation (Table 1, page 10). Both formulations contain 2 lb AI/gallon (240g AI/liter). The deposit evaluations included: in 1994, the Mimic 2F formulation; in 1995, the Mimic 2F and 2LV formulations; and in 1996, the Mimic 2F and 2LV formulations.

1994 — Ohio

Experimental area. Twelve plots, 16-135 acres (7-55 ha) in size, were selected in Columbiana County, Ohio. The plots were located in mixed oak, *Quercus* spp., stands on private lands and within an area of an increasing gypsy moth population. In the center of each plot, a 10-acre (4.0 ha) rectangular sub-plot was delineated and all treatment effects were measured within these sub-plots. Before and after treatment, egg mass (EM) counts were conducted using prism point and fixed-radius plots (Wilson and Fontaine 1978), 10 per sub-plot. A total of 50 egg masses were collected from the sub-plots in March and returned to the laboratory for eclosion in an effort to estimate overwintering mortality and incidence of NPV. Burlap bands were placed at breast height around 50 dominant or co-dominant oaks in each center 10-acre sub-plot and used as hiding or resting locations for gypsy moth larvae, pupae, and egg masses. The live larvae resting under the burlap bands were counted at weekly intervals for 4 weeks (i.e., L_1 is the first larval count), beginning 2 weeks after treatment. A count of live pupae was made during the L_4 count. The weekly percentage larval reduction was calculated from the average count per burlap per plot as follows: percent larval reduction = (1 – (treatment/untreated count)) x 100. Defoliation estimates were made on the burlapped trees in the center 10-acre sub-plot.

Deposit. For deposit assessment, a 10-acre area within 0.5 mile of one of the treatment plots was selected and treated using the same aircraft system, although the medium dose (0.06 lb AI/gal/acre) of the Mimic 2F + CS-7 tank mix was applied in the afternoon under marginal meteorological conditions (70°F, 40 % RH). Foliage was collected from 20 dominant white oaks, *Q. alba*, within the 10-acre sprayed area. Six leaves were obtained from the middle of the canopy from two different areas on each tree (three leaves from each area). The leaves were removed using 12 gauge shotguns, with number 4 shot. The fabric brightener Blankophor BBH (American Cyanamid, Princeton, NJ) was added at 0.2 percent w/v to the Mimic 2F + CS-7 tank mix to produce adequate fluorescence (Mimic 2F formulation contains UV protectants) when illuminated with a 70 watt long-UV lamp. A separate area was used for deposit assessment because of unknown effects of the optical brightener on the gypsy moth and non-target organisms. Leaves were photographed with Fujichrome ISO 100 slide film within 24 hours of collection. The drops on the photographs were sized and counted using a Swath Kit (Mierzejewski 1997). A spread factor was used to transform stain diameters to drop diameters. For deposit assessment within the treated plots, water sensitive and Kromekote cards were placed on the ground along one diagonal transect per plot prior to application. Following application the droplet deposit on these cards were not analyzed but recorded as "deposit" or "no deposit."

Non-targets. Evaluations were also conducted on three plots per treatment to determine the impacts of tebufenozide on non-target arthropods in the canopies of oaks and black cherry, *Prunus serotina* Ehrh. (Butler et al. 1997b). Pole pruners were used to remove foliage samples.

Field and laboratory studies were conducted to determine impacts of tebufenozide on several species of aquatic invertebrates inhabiting streams. Field studies included before and after, upstream and downstream quantitative sampling of aquatic macroinvertebrates in three streams draining two treatment plots (0.06 lb AI/acre). In the laboratory, Mimic 2F treated maple leaves were fed to six selected leaf shredding macroinvertebrates collected from local streams, and survivorship curves were compared between untreated leaves, field dosed leaves and two laboratory dosed leaves (≈ 0.03 and 0.06 lb AI/acre) (Stout 1996). The shredder aquatic macroinvertebrates included the amphipod *Gammarus minus* and the isopod *Lirceus* sp. representing the Crustacea. Insects selected included larvae of the cranefly, *Tipula abdominalis*, the caddisflies *Pycnopsyche gentilis* and *Pycnopsyche scabripennis*, and the stonefly *Amphinemura delosa*.

Treatments. Tebufenozide as Mimic 2F (aqueous flowable) was applied in one application by a Cessna 188B (AgTruck) equipped with TeeJet nozzle bodies with 45-8004 flat fan tips directed straight down, on the mornings of 21 and 22 May 1994. The meteorological conditions during the time of spray over several days were variable, 46-65°F and 60-75 % RH. The fixed-wing aircraft flew at 120 mph (193.1 km/h), assigned a 75 ft (23 m) swath width, and when characterized with the Mimic 2F formulation produced a VMD of approximately 250 µm. Treatments were applied when gypsy moth larvae were approximately 75 percent second and 25 percent first instars and leaf expansion on oaks was about 35 percent. The final spray mix consisted of 2 or 4 fluid ounces (fl oz) of Mimic 2F diluted with water and Latron CS-7 added to improve spreading and binding of the formulation to foliage at 2 pints/100 gal (0.236 liters/378 liters) of tank mix for a total of 1 gallon per acre. Four replicates of three treatments were applied: (1) Mimic low dose 0.03 lb AI/ gal/acre (0.14 kg AI/3.78 liters/0.4 ha) + CS-7, (2) Mimic medium dose 0.06 lb AI/gal/acre (0.28 kg AI/3.78 liters/0.4 ha) + CS-7, and (3) untreated (Table 1, page 10).

1995 – Virginia

Experimental area. Twenty-four plots, each 50 acres (20 ha) in size, were selected in Highland County, Virginia. The plots were located in a mixed oak forest on the Highland Wildlife Management Area (State lands) and within an area of an increasing gypsy moth population. In the center 10 acres of each plot before and after treatment, EM counts were conducted as in 1994; but there were 20 (instead of 10) fixed-radius plots. Burlap bands were placed around 10 white oak, *Q. alba*, per the center 10 acres of each plot for collection of larvae, pupae and egg masses.

Deposit. One plot sprayed with the Mimic 2F + CS-7 and one with the Mimic 2LV + CS-7(aqueous suspension with a vegetable oil) tank mixes were used for foliage analysis. The actual spray plots were used for deposit assessment (while in 1994 an area close to the actual spray plots was monitored) based on the results of laboratory studies indicating that the brightener Blankophor BBH did not negatively impact gypsy moth larvae. Thirty dominant or co-dominant oaks were sampled for each spray formulation for deposit. The sampling began 2 to 3 hours after spraying was completed. A new technique was developed to photograph deposit on the leaves while exposing them to a UV light source. Leaf areas were determined by taking the adjusted images, printing the image, and measuring the leaf area on a digitizing table.

Treatments. The Mimic 2F and Mimic 2LV formulations of tebufenozide were applied in one application each using a Cessna 188B (AgTruck) equipped as in 1994. Spraying began on the morning of 20 May and continued through 26 May. Meteorological conditions during the time of spray over several days were variable, 50-70°F and 30-80% RH. Gypsy moth larvae were 60 percent second and 40 percent first instars and oak foliage expansion was approximately 25 percent. The final tank mixes consisted of 4 or 6 fl oz of Mimic 2F or 4 fl oz of Mimic 2LV diluted with water and Latron CS-7 added at 2 pints/100 gal of tank mix for a total of 1 gallon per acre. Six replicates of four treatments were applied: (1) Mimic 2F at the medium

dose (0.06 lb AI/gal/acre) + CS-7, (2) Mimic 2F at the high dose 0.09 lb AI/gal/acre (0.42 kg AI/3.78 liters/0.4 ha) + CS-7, (3) Mimic 2LV at the medium dose + CS-7, and (4) untreated (Table 1, page 10).

1996 – West Virginia

Experimental area. Six plots, 114-185 acres (46-75 ha) in size, were selected in Wetzel County, West Virginia. The plots were located in a mixed northern hardwood forest on private land and within an area of an increasing gypsy moth population. In the center 20 acres of each plot before and after treatment, EM counts were conducted within 16 1/40-acre sub-plots (Kolodny-Hirsch 1986). Twenty dominant or co-dominant trees (at least 15 oaks and the remainder maple *Acer* spp. and cherry *Prunus* spp.) were burlapped per plot. Defoliation estimates were recorded in 10 percent increments (e.g., 0, less than 10, 10-20) at each of the 16 sub-plots.

Deposit. A Cessna 188B (Ag Truck) equipped as in 1994 and 1995 was used to spray a medium dose of the Mimic 2F, Mimic 2F + CS-7, and Mimic 2LV + CS-7 tank mixes to a total of three plots located in Staunton, VA. The same sampling protocols were used as in 1995.

Larval collections. One hundred first or second instars were collected from the lower canopy of understory foliage per plot on 14 May (before treatment), 20 May (5 hours after treatment), and 24 May (after treatment). The larvae were returned to the laboratory, reared on artificial diet, and percent mortality recorded. These collections of early instars were initiated in an effort to collect efficacy data due to treatments with minimal interaction from naturally occurring NPVs and the gypsy moth fungus, *Entomophaga maimaiga* (Webb et al. 1994).

Treatments. Tebufenozide as Mimic 2F was applied in one application by turbine Air Tractor AT-400 (135 mph, 150 ft swath) equipped with 41-8010 flat fan tips directed straight down. The aircraft was equipped with a Satlock GPS unit to record flight lines within treatment plots. Three replicates of two treatments were applied: (1) Mimic medium dose (0.06 lb AI/gal/acre) and (2) untreated. The adjuvant CS-7 was not added to the tank mix due to lack of supporting data from Rohm and Haas Company concerning its effectiveness as well as health concerns for individuals handling this material. The treatments were applied the morning of 20 May with meteorological conditions of approximately 70°F and 82 % RH. Gypsy moth larvae were 5 percent second and 95 percent first instars, with oak and maple foliage approximately 10 percent expanded.

DATA ANALYSIS

Treatments, including untreated, were assigned by a randomized complete-block without replications design. The egg mass density before spray was used as the blocking criterion. Egg mass density estimates before spray were transformed to square roots ($\sqrt{x}$) for analysis. Two-way analysis of variance (ANOVA) was used. After treatment, effects based on egg mass and percent defoliation data were not transformed and were analyzed using two-way ANOVA and Tukey's w-procedure. The population trend was calculated to show the magnitude of the change in egg-mass densities, as the ratio of densities before and after treatment for each plot. Percentage control was calculated by the following modification of Abbott's formula: percentage control = 100(1-T/C) where T and C are the treatment and untreated trends. Data on numbers of larvae and pupae found under the burlap bands were transformed to square roots for analysis. Two-way ANOVA and Tukey's w-procedure was used. Significance was reported at the $\alpha \leq 0.05$ level.

RESULTS

1994 — Ohio

Treatments. Average egg mass densities (range 287-356 EM/acre) before treatment were not significantly different ($F=0.24$; $df=2,9$; $P=0.7882$) (Table 2, Page 10). Analysis of 50 randomly collected egg masses from

the experimental plots before eclosion indicated that the population was not healthy, as 60 percent of the egg masses did not hatch with equally poor hatch among treatments. The average egg mass densities (range 116-236 EM/acre) after treatment in both the untreated and treated plots were downward and not significantly different ($F=4.5$; $df=2,9$; $P=0.6484$). The trends observed for average egg mass densities after treatment did not differ significantly from each other ($F=0.51$; $df=2,9$; $P=0.6168$).

Analysis of relative mean larval densities and percentage larval reduction by week, mean pupal densities and egg masses per burlap band (Table 3, page 11) showed that the treatments did not have a significant effect. Defoliation estimates in both the treated and the untreated plots were low, and differences were not significant.

Non-targets. The results for impacts on canopy arthropods were reported by Butler et al. (1997b). Briefly, before treatment collections showed similar richness and abundance of arthropods for all treatments. No differences were seen among treatments for non-target arthropod richness and abundance excluding macrolepidoptera. For macrolepidoptera abundance, numbers were significantly higher in untreated versus medium dose plots (0.06 lb AI/acre) for the early sampling period in 1994, and in untreated versus medium and low dose (0.03 lb AI/acre) plots for the late sampling period in 1994 and early sampling period in 1995.

The results of aquatic field and laboratory studies for impacts on aquatics were reported by Stout (1996). Briefly, aquatic macroinvertebrate populations through the course of before application, application, leaf-fall and subsequent incorporation of canopy leaves into headwater streams were not significantly different in density, diversity and functional group composition of aquatic communities in three treated and three untreated streams. In laboratory feeding trials, there were no significant differences in the survival of six species of leaf-shredding macroinvertebrates exposed to various concentrations of Mimic.

Deposit. There was deposit on less than 5 percent of the water sensitive and Kromekote cards placed on the ground along one diagonal transect in each treatment plot (i.e., efficacy evaluation). In the 10-acre area established for evaluation of deposit, the numbers of droplets per unit surface area of foliage were very low, with droplet densities averaging 1.2 drops per cm^2 and volume averaging 1.0 nanoliters (nl) per cm^2 of leaf surface. Ninety-five percent of the trees received 1.0 or less drops per cm^2 with a maximum deposit of 5.0 drops per cm^2. Only 10 percent of the spray volume was recovered from the forest canopy. The droplet spectra were characterized by a volume median diameter (VMD) of 201 µm and a number median diameter (NMD) of 129 (Table 4, page 12).

1995 — Virginia

Treatments. Average egg mass densities (range 1391-1454 EM/acre) before treatment were not significantly different ($F=0.13$; $df=3,20$; $P=0.9388$) (Table 2, page 10). The average egg mass densities (range 14-303 EM/acre) after treatment in both the untreated and treated plots were downward and significantly different ($F=5.97$; $df=3,20$; $P=0.0045$). The average egg mass densities were not significantly different between the Mimic treatments. The trends observed in both the untreated and treated plots were downward although significantly different ($F=4.87$; $df=3,20$; $P=0.0106$). The trends for the treated plots did not differ significantly from each other. The percentages of control were not significantly different.

Analysis of relative mean larval densities and percentage of larval reduction by week (Table 3, page 11) showed that the treatments had a significant effect on the larval population for each week although the relationship was not consistent for both weeks. The mean number of egg masses per burlap band were not significantly different among treatments ($F=2.59$; $df=3,20$; $P=0.0811$).

Defoliation estimates in the treated blocks averaged 11 percent and were significantly different than in the untreated plots where estimates averaged 52 percent ($F=8.35$; $df=3,20$; $P=0.0006$).

Deposit. Deposits per unit surface area of foliage for both the Mimic 2F + CS-7 and Mimic 2LV + CS-7 tank mixes were approximately the same (average 4.8 and 4.4 drops per cm^2, respectively). The distribution of

droplet density by tree was similar between the two tank mixes. Seventy-three percent of the leaves received between 2.1 and 8.0 drops per cm^2 and 13 percent received more than 8.0 drops per cm^2 for the Mimic 2F + CS-7 tank mix. For the Mimic 2LV + CS-7 tank mix, 67 percent of the leaves received between 2.1 and 8.0 drops per cm^2 and 13 percent received more than 8.0 drops per cm^2. Only 13 percent and 20 percent of the leaves received less than 2.0 drops per cm^2 for the Mimic 2F + CS-7 and 2LV + CS-7 tank mixes, respectively. The Mimic 2F + CS-7 tank mix resulted in a higher number of trees that received VMD's above 300 μm than did the Mimic 2 LV + CS-7 tank mix. The higher VMD values recovered for trees sprayed with the Mimic 2F + CS-7 tank mix led to higher recovered volumes than for trees sprayed with the Mimic 2LV + CS-7 tank mix. The average volume recovery was 916 nl/tree for the Mimic 2F + CS-7 tank mix and 278 nl/tree for the Mimic 2LV + CS-7 tank mix. The Mimic 2F + CS-7 droplets appeared larger than the droplets for the Mimic 2LV + CS-7 tank mix, while the Mimic 2LV + CS-7 droplets took longer to dry (Table 4, page 12).

1996 — West Virginia

Treatments. Average egg mass densities (range 312-478 EM/acre) before treatment were not significantly different (F=1.82; df=1,4; P=0.2489). The average egg mass densities (range 230-432 EM/acre) after treatment were close to significantly different (F=7.07; df=1,4; P=0.0564). The trends observed for after treatment average egg mass density were not significantly different (F=4.39; df=1,4; P=0.1043) between the untreated and treated plots. Analysis of relative weekly larval densities per burlap band (Table 3, page 11) showed that the treatments did not have a significant effect on the larval population except for L_5 and L_6, however, pupal densities per burlap band were significantly different (F=20.04; df=1,4; P=0.0110).

Defoliation estimates in the treated blocks averaged 5 percent and in the untreated blocks 20 percent. The low defoliation in the untreated blocks was anticipated based on low EM densities before treatment.

Deposit. Deposits per unit surface area of foliage averaged 3.7 drops per cm^2 for the Mimic 2F tank mix, 5.5 drops per cm^2 for Mimic 2F + CS-7 tank mix and 6.9 for Mimic 2LV + CS-7 tank mix (Table 4, page 12). The distribution of VMD by tree (in μm) for each tank mix is shown in Figure 1. The Mimic 2LV + CS-7 and Mimic 2F + CS-7 tank mixes produced more droplets with VMD's less than 150 μm than the Mimic 2F without CS-7 tank mix (i.e., 80.4 %, 75.9%, and 37.3 % respectively). The Mimic 2F + CS-7 tank mix had only approximately 24 percent of droplets with VMD's above 151 μm compared to 63 percent for the Mimic 2F without CS-7 tank mix.

Larval Collections. The results of the collections of early instar larvae before and after treatment are shown in Table 5, page 12. The mean percent dead before treatment was 7 percent for the treated plots and 10 percent for the untreated plots. The mean percent dead after treatment was 80 percent for the treated plots and 20 percent for the untreated plots.

DISCUSSION

The 1994 efficacy results for the Mimic 2F + CS-7 tank mix applied at 0.03 and 0.06 lb AI/acre doses -- 8 and 45 percent control respectively -- were disappointing based on greater than 90 percent control for similar doses in laboratory studies. There were no significant differences detected between the treatments based on egg masses, larvae and pupae per burlap band, and on percent defoliation. These poor efficacy results for both treatments were not anticipated, as the meteorological conditions at the time of aerial application were within the acceptable range (Miller et al. 1995). Samples were taken from each tank mix and assayed against second instar gypsy moth larvae at the USDA-APHIS laboratory as well as analyzed using gas chromatography at Rohm and Haas (Spring House, PA). Both doses provided 98 percent mortality of second instar larvae with the deposit very resistant to simulated rainfall as well as providing the desired level of active ingredient. The extremely low droplet counts (average 1.2 droplets/cm^2) and low volume recovery on foliage in the 10-acre area established for evaluation of deposit was reflective of the marginal meteorological

conditions at the time of spray and loss to evaporation. The naturally declining gypsy moth population was due to extremely low temperatures (average low -4°F (-20°C), high 18°F (-8°C)) from 15 to 23, January 1994 that resulted in high mortality of gypsy moth egg masses above the snow line. This mortality prevented the anticipated level of defoliation in the untreated plots. The poor efficacy and deposit results in 1994 prompted the re-evaluation of the 2F + CS-7 tank mix at 0.06 lb AI/acre in 1995. In addition, since evaporation of the droplets might have been part of the problem in 1994, Rohm and Haas Co. provided an aqueous suspension formulation with a vegetable oil (Mimic 2LV) that had been used successfully in Canada to control spruce budworm. In the Canadian trials, the Mimic 2LV formulation provided greater deposit on coniferous foliage than the aqueous flowable formulation (Mimic 2F) as well as excellent population reduction.

In 1995, the Mimic 2F + CS-7 tank mix at 0.06 lb AI/acre was selected as the "standard" treatment. A high dose (0.09 lb AI/acre) of the 2F + CS-7 tank mix was also evaluated in an effort to determine a maximum dose for gypsy moth control. The percent control for the three treatments (average 91 percent for the 0.06 lb AI/acre treatments and 96 percent for the 0.09 lb AI/acre treatment) was as anticipated based on laboratory results. There were no significant differences detected in either population reduction or percent defoliation between the treatments although all were different from the untreated plots. Once again, a natural population collapse impacted the percent defoliation in the untreated blocks. The distribution of droplet density was similiar between the two tank mixes applied at 0.06 lb AI/acre and averaged 4 to 5 drops per cm^2. Since these data showed that the aqueous flowable formulation (2F) + CS-7 at the 0.06 lb AI/acre dose provided comparable efficacy as the 0.09 lb AI/acre dose and deposit comparable to the aqueous suspension formulation with a vegetable oil (2LV) + CS-7 , the Mimic 2F formulation at 0.06 lb AI/acre without CS-7 was selected for re-evaluation in 1996.

In 1994 and 1995, the adjuvant CS-7 was added to all formulations based on the recommendations of the Rohm and Haas Co. representatives, although the authors expressed concern about potential fire and health hazards for people handling this product (Material Safety Data Sheet) as well as lack of data to support its effectiveness in sticking and binding the formulation to foliage. The aqueous suspension with a vegetable oil formulation (Mimic 2LV) was not evaluated for efficacy in 1996 as it provided comparable efficacy to the Mimic 2F formulation and, in general, formulations containing oil have not been used during Federal and State Cooperative Gypsy Moth Programs due to potential problems with phytotoxicity, difficulty in cleaning mixing and spray equipment, and other environmental concerns (e.g., non-targets).

In 1996, field efficacy results were excellent with 99 percent control although again compromised by a natural population collapse. In anticipation of that problem, early instar larvae were collected both before and after treatment and provided the efficacy data to support the effectiveness (i.e., 20 percent mortality in control plots and 79 percent mortality in the treatment plots) of the Mimic 2F formulation.

In 1996, the 2F+ CS-7 and the 2LV + CS-7 tank mixes provided comparable percentages of droplets with VMD's less than 150 µm. Both formulations + CS-7 provided comparable droplets per cm^2 (5.5 and 6.9 droplets per cm^2). Similar results were recorded for droplet density and distribution of droplets for these formulations + CS-7 in 1995. The 1995 and 1996 deposit data support the results reported for deposit on coniferous foliage and with the excellent control provided in 1995 support the use of the 2LV formulation to control gypsy moth on broadleaved foliage.

We recommend one application of the Mimic 2F or Mimic 2LV formulation without CS-7 at 0.06 lb AI/acre in total volume of 1 gal/acre for control of gypsy moth populations based on efficacy and droplet deposition. Preliminary non-target data collected in the 1994 efficacy plots indicated that at 0.06 lb AI/acre there were no detectable impacts to selected aquatic shredders while impacts were detected for macrolepidoptera. Obviously, additional evaluations at lower dosages and volumes for both formulations will be needed in an effort to lessen non-target impacts to macrolepidoptera while maintaining maximum efficacy for gypsy moth.

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Table 1. Mimic (active ingredient tebufenozide) treatments, doses and rates aerially-applied over three years (1994, 1995, 1996).

Year	Location	Treatments	n	Dose (lb AI/acre)	Rate (gal/acre)
1994	Ohio	Mimic 2F + CS-7	4	.03	1
		Mimic 2F + CS-7	4	.06	1
		Untreated	4	--	--
1995	Virginia	Mimic 2F + CS-7	6	.06	1
		Mimic 2LV + CS-7	6	.06	1
		Mimic 2F + CS-7	6	.09	1
		Untreated	6	--	--
1996	West Virginia	Mimic 2F	3	.06	1
		Untreated	3	--	--

Table 2. Analysis of egg mass density before and after treatments with Mimic (active ingredient tebufenozide), over three years (1994, 1995, 1996).

Year	Treatment	Dose	Mean (± SD) (egg masses per acre)			% Control
			Before	After	Trend	
1994	Mimic 2F + CS-7	.03	356 (84.6)	173 (61.0)	.649 (.33)	8
	Mimic 2F + CS-7	.06	287 (76.2)	116 (40.3)	.391 (.11)	45
	Untreated	--	354 (72.3)	236 (90.5)	.706 (.21)	--
1995	Mimic 2F + CS-7	.06	1433.0 (235.8)	25.4 (16.4)	.016 (.011)	95
	Mimic 2LV + CS-7	.06	1413.5 (309.8)	30.2 (19.9)	.018 (.013)	95
	Mimic 2F + CS-7	.09	1453.9 (178.6)	13.8 (5.3)	.010 (.005)	97
	Untreated	--	1390.9 (413.5)	302.7 (100.7)*	.340 (.146)*	--
1996	Mimic 2F	.06	478.3 (115.6)	3.3 (1.8)	.007 (.004)	99
	Untreated	--	311.7 (46.9)	431.7 (230.1)*	1.49 (.706)	--

Mean differences are significant (,$P \leq 0.05$) from all other treatment means.

Table 3. Average number of live gypsy moth larvae, pupae and egg masses (EM) per burlap band, and defoliation after treatment with tebufenozide, Mimic, for three projects (1994, 1995, 1996)

Year	Treatment	Dose	Mean (± SD)								
			Larvae per Burlap						Pupae per burlap	EM per burlap	Mean % defol
			L_1[a]	L_2	L_3	L_4	L_5	L_6			
1994	Mimic 2F + CS-7 [%][b]	.03	4.7 (2.6) [58]	17.5 (8.8) [20]	8.8 (4.4) [52]	.43 (.04) [73]			12.3 (6.0)	2.2 (.98)	6
	Mimic 2F + CS-7 [%]	.06	5.6 (4.9) [50]	11.5 (9.5) [47]	7.4 (6.0) [60]	.81 (.30) [49]			10.2 (7.3)	2.0 (1.0)	6
	Untreated	---	11.2 (2.8)	21.9 (3.1)	18.3 (1.7)	1.6 (.38)			33.6 (4.2)	8.0 (.72)	8
1995	Mimic 2F + CS-7 [%]	.06	1.4 (.90) [86]	.23 (.18) [0]					1.9 (1.1)	.43 (.28)	18
	Mimic 2LV + CS-7 [%]	.06	.83 (.42) [91]	0 (0) [100]					.02 (.02)	.15 (.06)	9
	Mimic 2F + CS-7 [%]	.09	.47 (.22) [95]	.02 (.02) [91]					.03 (.02)	.05 (.05)	6
	Untreated	---	9.7 (4.7)	.23 (.08)					11.9 (2.4)	1.6 (.76)	52
1996	Mimic 2F [%]	.06	22.7 (14.1) [63]	27.0 (18.1) [77]	7.7 (6.7) [50]	23 (22.5) [0]	1.3 (1.3) [99]	.33 (.33) [99]	.33 (.33)		5
	Untreated	---	60.3 (34.9)	123.3 (102.3)	16.0 (6.2)	22.0 (12.1)	188.3 (59.3)	68.0 (23.3)	169.3 (84.6)		20

[a] Larval counts at weekly intervals for successive weeks

[b] [%] = weekly percentage larval reduction

Table 4. Deposit expressed as median drop size (VMD), drop density (no. drops per cm^2), and median number of drops (NMD) on foliage sprayed with tebufenozide, MIMIC, over three years (1994, 1995, 1996)

			Deposit/tree		
Year	**Treatment**	**Dose**	**Drop Size (VMD)**	**Mean No. drops per cm^2**	**Drop Number (NMD)**
1994	MIMIC 2F+CS-7	.06	201	1.2	129
1995	MIMIC 2F+CS-7	.06	310	4.8	--
	MIMIC 2LV+CS-7	.06	260	4.4	--
1996	MIMIC 2F	.06	186	3.7	128
	MIMIC 2F+CS-7	.06	245	5.5	98
	MIMIC 2LV + CS-7	.06	275	6.9	69

Table 5. Collections and laboratory rearing of early instar larvae from tebufenozide treated and untreated plots in West Virginia, 1996

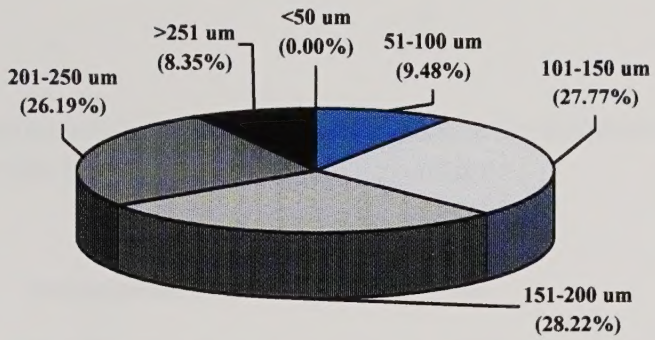
Pre-treatment data

Block	**T/C**	**n**	**5/21**	**6/4**	**6/11**	**Total**	**% Dead**
			Dead	***Dead***	***Dead***		
MP-1	T	92	4	2	1	7	7.6
MP-4	T	94	5	0	0	5	5.3
MP-5	T	89	7	0	1	8	9.0
MP-2	C	100	1	8	0	9	9.0
MP-3	C	97	9	2	0	11	11.3
MP-6	C	94	7	2	0	9	9.6

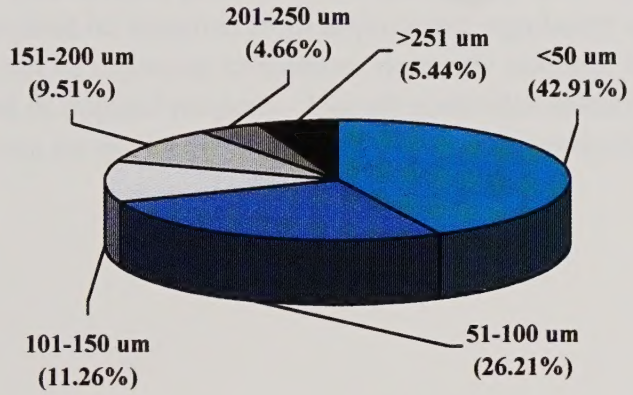
Post-treatment data

Block	**T/C**	**n**	**5/28**	**5/31**	**6/3**	**6/11**	**Total**	**% Dead**
			Dead	***Dead***	***Dead***	***Dead***		
MP-1	T	99	50	4	11	28	93	93.9
MP-4	T	98	24	2	20	24	70	71.4
MP-5	T	62	17	5	11	12	45	72.6
MP-2	C	87	2	6	2	17	27	31.0
MP-3	C	92	3	0	5	13	21	22.8
MP-6	C	98	1	0	1	6	8	8.2

Mimic 2F



Mimic 2LV + CS-7



Mimic 2F + CS-7

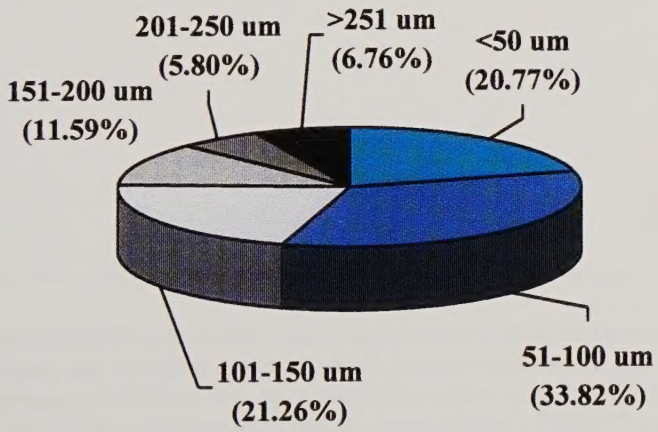


Figure 1. Distribution of Mimic (active ingredient tebufenozide) deposit on leaves in Virgina plots, 1996.

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